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T-LYMPHOCYTE EXCHANGE

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(57) Abstract

The invention concerns a T lymphocyte exchange method comprising for example the use of a T lymphocyte population for preparing a composition to be administered to a subject who has suffered a depletion of his T lymphocytes. The invention also concerns compositions and means for implementing said method. The invention is useful for treating numerous pathologies, in particular for controlling immunopathologies.

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This invention concerns the immunological and medical fields. More particularly, it concerns a new immunological approach allowing numerous pathologies to be treated. The invention is based more particularly on a "T-lymphocyte exchange" procedure with therapeutic purposes, following the depletion of the host's T-lymphocytes. The invention can be applied to the treatment of numerous pathologies in which T-lymphocytes are involved, such as autoimmune diseases, grafts, viral diseases, allergies, etc.

T-lymphocytes are cells that are essential in developing effective immune responses that allow the organism to be protected, in particular against infectious agents or tumor cells. However, although the T-lymphocyte response is usually beneficial, in certain situations it can be, on the contrary, the source of pathologies (autoimmune diseases, transplant rejections, etc.). In these situations, it is therefore especially important to be able to control the T-lymphocyte response in order to treat the immunopathology.

There are several treatments currently employed in order to control T-immunity responses when necessary. The treatment of the aftereffects of an organ transplant offers a good example of the use of these various treatments. Organ transplants are always followed by treatment by so-called conventional immunosuppressants, for example, Endoxan, corticosteroids, cyclosporin or FK506, aimed at protecting the transplanted organ from the host's immune response that can lead to rejection. Although these treatments have proven to be relatively effective, as proven by the progress made in organ transplantation, they do not always prevent transplant rejection. On the other hand, these treatments have other drawbacks: (i) they only "freeze" the immune response and must therefore be continued permanently (stopping immunosuppressive treatments completely usually leads to the rejection of the organ even after several years), and (ii) they are wholly nonspecific and therefore also inhibit favorable immune responses during the entire duration of this treatment. This results in increased sensitivity to infections and to certain cancers. This situation compromises the vital prognosis of the patients.

When rejections not controlled by these immunosuppressive treatments occur, there are therapeutic options other than simply increasing the doses of the medications employed. For example, there is the use of antibodies that destroy T-lymphocytes: polyclonal serums or monoclonal antibodies that recognize molecules expressed on the surface of the T-lymphocytes (anti-CD3, anti-CD4). These treatments act by destroying all T-lymphocytes, both those responsible for rejecting the transplant and the totality of other T-lymphocytes, and therefore also present a lack of specificity, inducing a significant immunodepressant state in the subjects.

For certain pathologies of the autoimmune type, the autologous bone marrow transplant ("BMT") has appeared as a new therapeutic approach. The interest of these treatments is to precede the bone marrow transplant by a conditioning leading to the destruction of the immune system, then to transplant the patient with his or her own hematopoietic cells, in order to achieve

a reconstitution by naive T-lymphocytes. However, since these lymphocytes are autologous, they may be involved once more in the immunopathological processes which led to performing this treatment. In addition, the reconstitution of T-lymphocytes based on bone marrow cells appears, in adults, to have very low efficacy.

Allogeneic BMT is now proposed for some of these pathologies, such as severe autoimmune pathologies, with a view to reconstitute the patient with different lymphocytes which should not participate in immunopathological reactions. However, allogeneic bone marrow transplant is marred by a significant morbidity, due to GVH, which limits its use. In effect, the T-lymphocytes found in the hematopoietic transplanted organ and reinjected into a patient who is immunodepressed from the conditioning necessary for transplantation will be responsible for an attack directed against the host cells, also called a graft-versus-host reaction (GVH). This reaction is sometimes hard to control by the standard immunosuppressive treatments and is also sometimes fatal.

At present it is not legitimate to propose a treatment with a mortality rate higher than 10% to a patient presenting chronic articular problems, which could however evolve for several years or even several dozen years.

Controlling pathological immune responses therefore at this time depends primarily on nonspecific immunosuppressive treatments that do no more than temporarily "freeze" these responses. In spite of all the interest of these treatments, which have amply proven their clinical efficacy, it is now important to develop other therapeutic modes whose objective would be to achieve a more specific, and possibly permanent, immunosuppression.

This invention represents a new therapeutic approach for regulating immune response. More particularly, this invention concerns a new concept for modulating the T-lymphocyte immune response, especially the immunopathological T-lymphocyte response. The invention has numerous advantages compared with the approaches described in the prior art, especially in terms of specificity, stability (duration) and comfort for the subjects.

A first aspect of the invention concerns, more particularly, a method for modifying the immune system of a subject that involves replacing that subject's T-lymphocytes. More particularly, this method consists of depleting the T-lymphocytes (or T-lymphocyte subpopulations) of the subject (without depleting other hematopoietic cells, including stem cells), followed by the administration of a composition consisting of modified autologous, syngeneic or allogeneic T-lymphocytes (or T-lymphocyte subpopulations). This method is more particularly aimed at controlling immunopathological responses mediated by T-lymphocytes.

Another particular aspect of the invention also concerns a method for regulating immunopathological responses induced by T-lymphocytes, that consists of replacing all or part of a subject's T-lymphocytes by a population of modified T-lymphocytes.

The invention also relates to a method for treating pathologies induced by T-lymphocytes, that involves replacing all or part of a subject's T-lymphocytes by a composition consisting of modified T-lymphocytes.

The invention also concerns the use of a T-lymphocyte population for preparing a composition meant to be administered to a subject who has undergone a depletion of all or part of his or her T-lymphocytes.

The invention also concerns the preparation of a composition of T-lymphocytes whose repertoire has been modified (enriched by or, inversely, depleted of, certain antigenic characteristics).

This invention therefore concerns a new medical approach, based on replacing a subject's T-lymphocytes, often referred to hereinafter by the expression "T-lymphocyte exchange."

This invention is based in part on a new concept of T-lymphocyte homeostasis. In particular, the invention is based on the idea according to which, in adults, T-lymphocyte homeostasis occurs essentially based on movements of mature T-lymphocyte populations and not, as has been thought up to now, based on the differentiation of hematopoietic stem cells with T-lymphocyte differentiation via the thymus.

The set of facts (i) regarding the reconstitution of T-immunopoiesis following bone marrow transplantation based on purified immature hematopoietic stem cells (depleted of lymphocytes and/or purified with regard to CD34), (ii) regarding immunopoiesis following bone marrow transplant in terms of age, and, finally, (iii) regarding T-lymphocyte homeostasis during HIV infection, supports this new concept.

These facts are also consistent with the set of current facts concerning T-lymphocyte homeostasis in mice. In particular, it has been reported that a mouse thymectomized more than three days after birth lives normally with a normal number of T-lymphocytes. On the other hand, a mouse thymectomized before D3 does not develop T-lymphocytes, but if mature T-lymphocytes are reinjected into it, they develop, and the mouse lives normally with a normal number of T-lymphocytes.

The principle of the invention is therefore particularly novel because it proposes that the replacement of a patient's T-lymphocytes does not necessarily have to occur through bone marrow or hematopoietic stem cell transplantation (preceded by medullary aplasia (conditioning)), but that it can be performed by a less invasive method, which is the subject of this invention, to wit, the "T-lymphocyte exchange."

The invention therefore offers a first advantage compared to the previous technique, which lies in the rapid reconstitution of immunity. Thus, according to this invention, the T-lymphocytes injected into a patient allow a much faster expansion than the reconstitution based on T-lymphocytes that come from the differentiation of hematopoietic cells, which leads to

achieving the complete (or nearly complete) replacement of the patient's T-lymphocytes by a pool of T-lymphocytes that come from the injected ones.

The invention has another important advantage compared to the previous techniques, which lies in the characteristic of specificity. Thus, unlike the standard techniques using immunosuppressants or BMT's, which are preceded by complete medullary aplasias, this invention:

- offers specificity in relation to immunopathological T-lymphocytes, and
- only modifies part of the subject's immune system.

Finally, the invention is also based on the destruction of immunopathological T-lymphocyte clones, and thus also has the characteristic of stability to the degree that the clones are definitively suppressed.

This invention thus offers numerous advantages compared to the previous methods, and has multiple applications.

The results described above, which indicate the existence of an autonomous and homeostatic compartment containing T-lymphocytes, are the basis of a general principle for treatment by mature T-lymphocytes, said treatment consisting of at least 3 operations, which may, if necessary, be separated in time:

1) the obtainment of a sample of autologous, syngeneic or allogeneic T-lymphocytes, with the donor having T-lymphocytes that present immunity, antigenicity, age or other desirable characteristics to be conferred to the recipient,

- where appropriate, the genetic transformation of the lymphocyte sample in order to confer a given property,
- where appropriate, the modification (enrichment, depletion, impoverishment, etc.) of this lymphocyte population in a particular antigenic subclass, by in vitro or ex vivo depletion, enrichment or impoverishment, for example, by immunoaffinity,

2) the destruction of all or part of the T-lymphocytes of the recipient patient,

3) the injection of said enriched, depleted, transformed or untransformed lymphocytes to the recipient after the total or partial destruction of his or her own lymphocytes.

The population of donor lymphocytes, where appropriate genetically transformed or enriched by depletion of one or several not rare subclasses, may be extemporaneously administered to the recipient or frozen, for example, by the technique described in patent application number PCT/FR 97/00385.

Freezing allows the consideration of a long-term treatment with a product which remains perfectly homogenous over time.

In the same way, the destruction of all or part of the patient's T-lymphocytes is not necessarily required in all the applications of the technique.

The applications of this type of treatment are numerous and cover all the pathologies or physiological changes involving the immune system. In particular, this type of treatment may be applied to autoimmune diseases, to allergies, to the aging of immune functions, such as anergies, etc.

This invention has at least two characteristics:

- the total or partial replacement of a patient's T-cells,
- the total or partial destruction of pathological clones which are responsible for GVH and/or a preexisting pathology, such as autoimmunity, and/or, more generally, which present poor regulation of the immune system. This destruction is performed by a system that specifically destroys the activated clones of the reinjected cells at the chosen time. This destruction may be performed by any method chosen by those skilled in the art, as detailed below.

More specifically, a first purpose of the invention concerns the use of a population of T-lymphocytes to prepare a composition to be administered to a subject who has undergone T-lymphocyte depletion.

This invention is therefore based, on the one hand, on the depletion of the recipient's T-lymphocytes, and, on the other hand, on the administration of a composition of T-lymphocytes. A general diagram of the "T-lymphocyte exchange" is shown in Figure 1.

As shown by this figure, the T-lymphocyte population may include T-lymphocytes that are autologous, syngeneic or allogeneic in relation to the subject.

The term "autologous" designates a population of cells that come from the subject to whom they are administered. In this embodiment, the T-cells are therefore extracted from the subject before the depletion is performed.

The term "syngeneic" designates a population of cells that come from an identical twin of the subject to whom they are administered.

The term "allogeneic" designates a population of cells that come from another subject of the same species (related or not), that is, from another human being in the case of the treatment of a human patient.

The T-lymphocyte population may be obtained and prepared by any technique known by those skilled in the art. Thus, the lymphocytes may be isolated from a subject's blood, lymphoid organs or others by the standard techniques (cytapheresis, density gradients, cellular Tri, etc.) or they may be obtained from stock banks. For a sample from a subject, the lymphocytes are preferably obtained from peripheral blood mononuclear cells (PBMC) or from umbilical cord blood. Umbilical cord blood is an interesting source. In effect, the T-lymphocytes of the umbilical cord have the advantage of including a very diversified repertoire and being able to withstand numerous divisions.

When a population of autologous T-lymphocytes is involved, they may, therefore, in a particular embodiment of the invention, be taken from the subject himself or herself (or from his or her true twin, if any) by cytophoresis from PBMC's or umbilical blood.

When a population of allogeneic T-lymphocytes is involved, they may, in a particular embodiment of the invention, be taken from any allogeneic donor, preferably chosen because of HLA compatibilities with the recipient, so that there may be cooperation between the recipient's cells with antigens and the donor's T-lymphocytes. In certain situations, the T-lymphocytes may be taken from a donor chosen because of a particular characteristic (for example, his or her proven capacity to respond to an infectious agent).

In order to perform the T-lymphocyte exchange according to the invention, it is preferable for the T-lymphocyte population employed to be composed of T-lymphocytes representing a diversified repertoire. In effect, this allows favoring a rapid reconstitution of the subject's immunity, thus avoiding the persistence of a significant immunodepression in the subject. In this regard, it is calculated that the peripheral blood contains approximately 2% of all the mature T-lymphocytes of a subject. In addition, it is considered that, for a given specific antigen, the frequency of the specific T-lymphocyte clones is approximately 1 out of 10^5 cells. Thus, based on approximately 10^8 T-lymphocytes from the peripheral blood, there is a T-lymphocyte population in which each antigenic characteristic is represented by approximately 1000 cells. A population of this size therefore allows a perfectly satisfactory representation of the T-lymphocyte repertoire. Preferably, when obtaining the sample, it is advisable to obtain a T-lymphocyte amount on the order of 10^6 to 10^9 , preferably 10^7 to 10^8 cells approximately, so as to cover a sufficient immunological repertoire. Subsequently, if necessary, these cells may be cultured and developed in vitro (ex vivo). Preferably, the T-lymphocyte population used for administration consists of approximately 10^7 to 10^{12} T-cells, even more preferably between 10^8 and 10^{11} , advantageously 10^9 to 10^{10} T-cells. It is understood that these amounts may be adapted by those skilled in the art.

In addition, the T-lymphocyte (T-Ly) population used may include certain cells of a different nature (non-T-Ly). Thus, the T-lymphocyte population advantageously is composed of at least 60% T-Ly's, preferably at least 80%, even more preferably at least 95% T-Ly's. The other cellular types present may be, for example, B-lymphocytes or other blood cells, generally hematopoietic ones. The quality of the T-lymphocyte population may be determined by any technique known to those skilled in the art, especially by marking with different specific antibodies, and analyzing by flow cytometry. In addition, the quality of the lymphocytes may also be verified by analyzing their repertoire by flow cytometry or by the immunoscope technique.

Within the framework of this invention, the lymphocyte population used is conventionally a population modified to improve the immune properties of the subject. The modification or modifications provided to the T-cells may be of various natures.

Thus, the modification(s) may be of a genetic or immunological order, for example.

In a first particular variant of the invention, the T-lymphocyte population used consists of genetically modified T-lymphocytes.

The term "genetically modified" indicates that the lymphocytes contain a nucleic acid that is not naturally found in lymphocytes in their nonmodified state, or a nucleic acid that is found in a nonnatural state in lymphocytes (an artificially extended sequence, for example). Hereinafter, this nucleic acid shall be called a heterologous nucleic acid.

More preferably, the genetically modified lymphocytes used within the framework of this invention consist of a heterologous nucleic acid consisting of a suicide gene. In terms of the invention, the expression "suicide gene" is more particularly understood to mean any nucleic acid coding for a toxic product, that is, for a product (RNA, protein, etc.) that is capable of inducing the destruction of the cell that contains it, by any mechanism. Preferably, it is a nucleic acid coding for a product (for example, a protein) that has a conditional toxicity, that is, which is capable of transforming a normally inactive drug into a metabolite that is highly toxic to the cell. In this type of toxicity, although all the genetically modified cells express the suicide gene, cellular toxicity is strictly controlled by the administration of the drug.

In a particularly preferred embodiment, the nucleic acid used (that is, the suicide gene) is a nucleic acid whose toxic action specifically affects dividing cells. Examples of this type of nucleic acid include thymidine kinase (TK), among others.

The suicide gene most frequently used, and which seems to have the most interesting biological properties for controlling cellular immune responses, codes for the thymidine kinase of the type 1 *Herpes simplex* virus (HSV1-TK). This enzyme, in contrast with cellular thymidine kinases, is able to phosphorylate different nucleosidic analogues, such as acyclovir (ACV) or gancyclovir (GCV) into monophosphate derivatives which are then transformed into di- and triphosphate compounds by cellular enzymes. These triphosphate compounds can then be incorporated into the DNA by cellular polymerases during elongation. This incorporation induces a termination of the elongation and triggers apoptosis of those cells [11-18]. Thus, the toxic doses of GCV necessary for destroying HSV1-TK-expressing cells are at least 100 times less than those necessary for destroying parenteral cells (approximately 10 times for ACV). Since GCV is much more effective than ACV in this system, it is the drug most frequently used, although it requires administration by perfusion twice daily. The induction of cellular death by blocking DNA elongation implies that only dividing cells are affected by GCV, which has been experimentally confirmed. Thus, the HSV-1 TK gene has been used for genetic cancer therapy

[5,6], and, more recently, for destroying T-Ly's at the beginning of pathological immune responses [1,2,7-9].

This system therefore has three particularly interesting characteristics for this invention: (i) a conditional toxicity that depends on the administration of a drug (a nucleoside analogue) which allows the T-Ly destruction period to be controlled; (ii) a toxicity that is restricted to dividing cells, belonging to activated T-Ly's; and (iii) a great flexibility of use that allows the use of various drug administration regimes.

The prototype of the suicide gene is the HSV1-TK gene that works with gancyclovir.

Other thymidine kinases can also be used, such as the thymidine kinase derived from type IV equine herpes viruses. Truncated TK genes can also be used, such as the TK delta gene or an enzyme of human origin mutated so that it acquires the properties of these viral enzymes.

In this regard, several teams began therapeutic trials based on the transfer of an HSV1-TK gene to mature T-Ly genes which are then reinjected during leukemia treatment by allogeneic BMT [27,28]. The first known results of these studies indicate that it is possible to control the reactivity of these T-Ly's in vivo by a gancyclovir (GCV) treatment [7]. The experiments performed on animals and human beings therefore show that it is possible to control GVH after allogeneic bone marrow transplantation if the T-Ly's injected are HSV1-TK gene carriers. However, the proposed operating method is still extremely unwieldy since it involves replacing all the hematopoietic lines of the recipient.

The use of a nucleic acid coding for a conditional toxin constitutes a particularly preferred embodiment of the invention. In effect, the presence of this type of construction in the lymphocytes used for the administration allows the T-immunopathological responses to be specifically controlled. Thus, in the case of autologous T-lymphocytes, the presence of the suicide gene is used to destroy the T-lymphocytes engaged in pathological immune responses and thus to suppress, in a lasting manner, the lymphocyte clones responsible for these pathologies. In the case of allogeneic T-lymphocytes used within the framework of bone marrow transplantation, the presence of the suicide gene is used to destroy T-lymphocytes engaged in a graft-versus-host response, thus avoiding the development of this type of pathology.

Thus, in an example of the application of this invention, a patient affected by a T-immunopathological response-mediated pathology is subjected to an effective T-lymphocyte depletion (as described further below), and is then reconstituted with T-lymphocytes. These T-lymphocytes may be either the T-lymphocytes of the patient himself or herself (autologous) after they have been transduced by a suicide gene (that is, genetically modified), or similarly modified allogeneic T-lymphocytes. The mature T-lymphocytes thus injected into the subject whose own mature T-lymphocytes have been depleted will proliferate and expand rapidly in order to reconstitute the pool of mature T-lymphocytes following the homeostatic property of the

mature T-lymphocytes of undergoing an *in vivo* expansion such that their number achieves predepletion values. In this respect, the rare T-Ly's remaining in the subject will be able to multiply even less since the compartment dedicated to the T-Ly's will have been "filled" with the injected cells. Then, after the pathological phases, the subject is treated by a drug that can be transformed into a toxic metabolite by the suicide gene employed, which leads to a preferential elimination of the dividing T-lymphocytes alone, that is, only T-lymphocytes which are activated and therefore responsible for the pathology. This T-lymphocyte exchange and subsequent treatment in the presence of the drug allows the subject's lymphocytic repertoire to be modified *in vivo*, in a lasting (or even permanent) manner.

In addition, the invention has the advantage of being able to be implemented, even without prior knowledge of the antigens involved in developing the pathology in question.

According to another variant of the invention, the T-lymphocyte population employed consists of immunologically modified T-lymphocytes. More particularly, this immunological modification consists of the *in vitro* (or *ex vivo*) modification of the T-lymphocyte repertoire of said population. More preferably, this immunological modification consists of the *in vitro* or *ex vivo* suppression of lymphocyte clones involved in the immunopathological responses and/or of T-lymphocyte subpopulations having particular immunological properties (CD4+ or CD8+, TH1 or TH2, etc.).

Advantageously, the T-lymphocyte population used therefore consists of a "hole" in the immunological repertoire.

This embodiment is particularly adapted to situations in which the antigen or antigens recognized by the immunopathological clones are known. In effect, in this case, it is possible to perform an *in vitro* or *ex vivo* depletion of the T-lymphocyte population by immunoaffinity by means of said antigens or of fragments or homologues of the same, for example. One such depletion may be performed, by example, by putting the T-lymphocyte population in contact with a support on which said antigens or fragments or homologues are immobilized. The depletion of the clones recognizing specific antigens involved in the immunopathology may also be performed by a transfer of suicide genes to the T-lymphocytes, if these are then stimulated *in vitro* in the presence of the antigen and of a conditional toxic treatment (GCV). In the absence of a genetic modification, these lymphocyte clones activated by the antigen *in vitro* may also be destroyed by antibodies coupled with toxins which specifically recognize the molecules expressed by the activated T-lymphocytes (II2 receptors).

This embodiment may be performed, for example, in the case of pathologies such as organ transplantation in which the organ donor's alloantigens are involved.

This immunological modification of the T-lymphocytes may be implemented either with an autologous or syngeneic population, or with an allogeneic population. In addition, it may be combined with a genetic modification as described above.

Thus, in a particular embodiment, the T-lymphocyte population used consists of lymphocytes that are modified:

- immunologically, by depleting specific clones and/or subpopulations of T-lymphocytes with particular immunological properties (CD4+ or CD8+, TH1 or TH2, etc.), and
- genetically, by introducing a nucleic acid coding for a toxic product.

According to another variant of the invention, the T-lymphocyte population used consists of allogeneic T-lymphocytes giving increased immunity. In particular, it is possible to use T-lymphocytes from a subject having an appropriate (pronounced or increased) response to infectious agents. Thus, it is known that the T-immunity response is partially controlled by very diverse genetic factors. Because of this, with respect to a given antigen, certain subjects are able to show appropriate (beneficial) responses while others are not. According to the invention, it is therefore possible to replace the T-lymphocytes of a subject who is sensitive to a given pathology with those of a subject who is not sensitive to it.

In this embodiment, it is not necessary for the T-lymphocytes to be immunologically modified. Nevertheless, it is preferable, in order to be able to control all graft-versus-host reactions, for these T-lymphocytes to be genetically modified as described above.

In addition, in a particular embodiment of the invention, it is possible to use a T-lymphocyte population such as described above which is genetically and/or immunologically modified and/or giving increased immunity and which also consists of a nucleic acid coding for a therapeutic product. In effect, according to this invention, it is also possible to confer a new property on the T-lymphocytes employed. Since T-lymphocytes are cells which can be easily obtained, isolated and cultured, they may in effect be used simultaneously as a source for introducing (or reintroducing) proteins, or absent or abnormal functions, in a subject.

Various approaches may be used for the genetic modification of the T-lymphocytes, according to the techniques of those skilled in the art.

In a particular embodiment of the invention, the lymphocytes are genetically modified by means of a viral vector. In this embodiment, the heterologous nucleic acid is, for example, introduced in a viral vector, which is then used to infect a T-lymphocyte population as described above.

Different types of viral vectors may be used, especially retroviral or AAV vectors. In a preferred embodiment, the lymphocytes are genetically modified by means of a retroviral vector.

Retroviruses are the vectors of choice for transferring genes to T-lymphocytes because retroviral infection leads to the stable integration of the genome in the cells. This is a very

important property, taking into account the fact that lymphocytic expansion, either in vitro or in vivo after injection in the subject, supposes that the transgene is stable throughout segregation to be transmitted to each cellular division. With regard to the types of retroviruses that may be used, for example, the retroviruses belonging to the oncovirus, lentivirus or spumavirus families may be mentioned. Within the oncovirus family, especially the slow, nononcogene-bearing oncoviruses may be mentioned, such as, for example, MoMLV, ALV, BLV or MMTV, and the rapid oncoviruses, such as RSV, for example. Within the lentivirus family, for example, HIV, SIV, FIV or CAEV may be mentioned.

Techniques for constructing defective recombinant retroviruses have been widely described in the literature (WO89/07150, WO90/02806, WO94/19478, etc.).

In a particular embodiment of the invention, a recombinant retrovirus consisting of a GALV virus envelope (GALV-pseudotyped retrovirus) may be used to advantage. In effect, it has been shown that infecting hematopoietic cells with a recombinant retrovirus is achieved more effectively when the retroviral envelope is derived from a retrovirus called Gibbon Ape Leukemia Virus (GALV) (Movassagh et al., Hum. Gene Ther. 9 (1998) 225). With the aid of this retroviral envelope, we have further shown that it is possible to obtain transduction rates greater than 95% in mature T-lymphocytes before any transduced cell selection (unpublished results).

On the other hand, the recombinant virus used may contain expression-regulating sequences (promoters) specific to certain T-lymphocyte subpopulations. Thus, in certain situations, it may be advisable not to express nucleic acid except in certain T-cell populations, such as, for example, CD4+ or CD8+ cells, or in the so-called Th1 or Th2 cells for which specific markers have been described in mice, allowing their separation. Since these cells are characterized by the type of cytokines they produce (for example, IL2 or IFN γ for Th1, and IL4 for Th2), it is possible to use the sequences regulating these various genes to control the expression of heterologous nucleic acid (especially of the suicide gene). It is also possible to use promoters controlling a specific expression in a given T-lymphocyte population, such as, for example, the promoter coding for the CD4 molecule.

In addition, in another particular embodiment of the invention, the lymphocytes are genetically modified by means of a retrovirus produced in an encapsidation line consisting of a truncated Pol gene.

One of the risks associated with using viral vectors for transferring genes is the possibility of generating replicating viruses. The use of so-called separated genome lines has allowed this phenomenon to be limited. It is possible to minimize it even more by using constructions specifically known for decreasing recombination events as much as possible. We have shown, for example, that truncating the 3' end of the Pol gene at the SmaI enzyme cutting site on the ecotrope Moloney retrovirus genome (PNCA) allows the polymerase function to be wholly

preserved while suppressing all of the overlapping sequences between the Pol gene and the gene coding for the Moloney virus envelope. The use of this type of construction to make recombinant retroviruses capable of transducing the T-lymphocytes thus allows an improvement in terms of safety.

The infection of T-lymphocytes by retroviral vectors may be performed according to any technique known to those skilled in the art (incubation with a retrovirus supernatant, retrovirus encapsidation T-cell lymphocyte coculture, Transwell techniques, etc.). A particularly effective method has been described by Movassagh et al. (cf. above), which consists of a centrifugation stage that may be repeated. This method can be advantageously implemented to genetically modify T-lymphocytes according to the invention.

For a better implementation of this invention, it is suggested to obtain the most effective gene transfer possible in the T-lymphocytes. Thus, it is preferable to use a T-lymphocyte population consisting of at least 50%, preferably at least 65%, more preferably at least 80% genetically modified T-lymphocytes. In an ideal embodiment, a T-lymphocyte population is used whose genetic modification rate is as close as possible to 100%.

As indicated above, such levels may be obtained in vitro or ex vivo, using, for example, a GALV envelope, and, if necessary, under certain conditions of infection (Movassagh et al., above). In addition, to the degree that the genetic modification is performed in vitro, it is also possible to improve transduction levels by selecting effectively infected cells. For this, various selection techniques known to those skilled in the art are available, especially the use of antibodies that recognize specific markers on the surface of infected cells, or the use of resistance genes (such as the neomycin and G418 drug resistance gene), or even compounds toxic to cells that do not express the transgene (that is, thymidine kinase). In this respect, it would be possible to use a nontoxic molecule only for HSV1-TK gene-bearing cells. The method for finding this molecule is to screen with a library of molecules known for their toxicity, and to test those that are 1) modified by the HSV1-TK enzyme and 2) whose metabolites are not toxic. The advantage of such a method is that there is no need to transfer a second gene, and the selection only applies to undesirable cells.

Preferably, the selection is made using a marker gene, inserted into the retroviral vector, which expresses a membrane protein. The presence of this protein thus allows selection by standard separation methods, such as Tri by magnetic balls, columns or Tri by flow cytometry. From this perspective, a gene such as the one coding for the human Thy1 molecule is advantageous. The use of a molecule such as CD34 may also be considered, which is missing from mature T-Ly's, because there are already cell selection systems bearing this surface molecule, such systems having already been validated by cellular Tri and used clinically. In addition, these markers can also include tag sequences, for example, the c-myc tag.

The use of a membrane expression marker gene has two additional advantages within the framework of this invention: (i) it allows the genetically modified T-lymphocytes to be followed easily when they are injected, and, especially, (ii) it allows their destruction (even in the absence of division) due to the action of an antibody specifically directed against this molecule (if it proves to be necessary).

Advantageously, in order to express the marker gene (such as Thy-1), the use of vectors, known as bicistronic vectors, for which the toxic genes (for example, HSV1-TK) and the marker (for example, Thy1) are separated by an IRES sequence, is preferred.

The T-lymphocyte population obtained, if need be, genetically and/or immunologically modified, and/or having increased immunity, may be conditioned in any appropriate medium and device. Thus, the usable media are all mammalian cell culture media (RPMI, DMEM, etc.) or any other solution appropriate for preserving and/or storing mammalian cells (saline solutions, buffers, etc.). The device used may be, for example, a tube, flask, box, phial, syringe, bag, etc., preferably in a sterile condition appropriate for pharmaceutical use. The cellular composition may be used extemporaneously or it may be stored (refrigerated, frozen or lyophilized, for example) for later use. In addition, as indicated further below, banks of such cells may be advantageously made, under the preparation and storage conditions described above.

As indicated above, the invention consists of exchanging a subject's T-lymphocytes, and this includes the administration of mature T-lymphocytes to a subject who has undergone a depletion of his or her own T-lymphocytes.

Before administration, the subject is therefore subjected to a stage during which his or her existing T-lymphocytes are depleted. It is understood that when the T-lymphocyte population employed is an autologous T-lymphocyte population, this population is prepared before the depletion.

Depletion may be performed in various ways. Thus, when there is concomitant bone marrow transplantation, depletion may be performed by the conditioning, including radiotherapy.

Nevertheless, depletion is more preferably performed by treating the subject with one or more immunosuppressive agents, in particular a T-lymphocyte-specific immunosuppressive agent. Thus the subject is not subjected to total bone marrow aplasia and retains a certain immunity, in spite of the T-lymphocyte immunosuppression.

Preferably, the immunosuppressive agent used is an "antilymphocytic serum," or one or more monoclonal antibodies specific to T-lymphocyte surface molecules. In particular, it is possible to use a serum or anti-DC3, CD4 and/or CD8 antibodies. This type of immunosuppressant (serum or depleting antibodies) has already been used in therapy. Thus, experiments performed on mice and human beings show that it is possible to deplete the mature T-lymphocytes of an animal or a patient very effectively by means of these treatments (Muller

et al., Transplantation 64 (1997) 1432; Caillat-Zucman et al., Transplantation 49 (1990) 156).

It is not necessary for the mature T-lymphocyte depletion to be complete for the T-lymphocyte exchange according to the invention to be effective. Thus, even if a certain percentage of T-lymphocytes remains, it is unlikely that they will suffice to reinduce the immunopathology. Generally, the immunosuppressive treatments described above lead to a depletion greater than 90%, often as great as 95%, which is perfectly appropriate for this invention.

The T-lymphocyte composition may be administered in various ways and according to various protocols. It is advisable to administer the T-lymphocytes at a date such that the treatments administered to the patients in order to destroy their own T-lymphocytes have been eliminated, so that they cannot in turn attack the T-lymphocytes to be injected.

Preferably, the composition is administered a short time after depletion (for example, less than 48 h later), so as to favor the spread of the mature T-lymphocytes injected, which will have a natural tendency to fill the mature lymphocyte "hole." In addition, the treatments may be repeated if needed, with the same cells, or even the cells of different donors, but following the same therapeutic principle. The administration may be advantageously performed by intravenous or intraarterial injection.

As indicated above, the doses administered are generally between 10^8 and 10^{11} T-lymphocytes per subject.

In addition, during the procedures described above for preparing T-lymphocyte populations, it is possible to preserve the patient's cells (normal or transduced by the suicide gene), which can be used in case of need (stocks 1 and 2 in Figure 1). For example, if undesirable effects occur, it would still be possible to deplete the re-injected (for example, allogeneic) cells in order to then re-inject the patient's (normal) cells.

Likewise, in the case of an allogeneic exchange, the (transduced or normal) donor cells may also be preserved for further use if needed (stocks 3 and 4 in Figure 1). Moreover, it is possible, in a patient who has received GVH-controlled transduced allogeneic T-lymphocytes, to inject normal lymphocytes from the same donor without GVH (active tolerance). This would have the advantage of reinjecting cells which have not been cultured. Thus, advantageously, the procedure of the invention also includes a stage of constitution of banks of T-cells, which may or may not be transduced, and which come from the donor and the recipient.

Following the administration of the compositions described above, the invention allows the emergence or development of immunopathologies to be controlled, either by administering the drug, metabolized into a toxic product (in the case of T-lymphocytes which are genetically modified by a toxic gene), or simply by constituting an increased immunity in the subject.

Another purpose of the invention also lies in a product consisting of:

- one or more immunosuppressive agents, and
- a composition consisting of a T-cell population such as defined above,

with a view to a use that is separated or spaced in time, especially for controlling immunopathological responses.

Preferably, the composition consists of a diversified T-lymphocyte repertoire.

Advantageously, the composition consists of a T-lymphocyte population consisting of a suicide gene, and the product of the invention in addition consists of a drug which can be transformed into a toxic metabolite by the suicide gene.

The invention also concerns a technique for preparing a composition of modified T-lymphocytes consisting of:

- obtaining T-lymphocytes from a subject, and
- creating a hole in the lymphocyte repertoire or, more generally, selectively modifying the repertoire.

More particularly, the hole in the repertoire is performed by depleting T-lymphocytes specific to antigens involved in pathologies.

The invention also concerns all the T-lymphocyte populations obtained from a patient, which have been depleted of the clones of specific T-lymphocytes or of one or more antigens involved in one or more pathologies.

In addition, the invention can also be implemented with any other type of compartmentalized cell, that is, any type of cell meeting the following criteria:

- cells of which samples may be taken, whose entire population may be destroyed and which may be (re)injected (with modifications) and take the place of those that have been destroyed.
- for example, a subpopulation of T-Ly's or other circulating cells (satellite muscle cells) may be considered.

The applications of the invention are extremely numerous. They especially concern, both for autologous and allogeneic T-lymphocytes modified according to the invention, all the autoimmune and chronic inflammatory pathologies in the general sense (rheumatoid polyarthritis, multiple sclerosis, etc.), viral infections (hepatitis A, B, C, HIV, etc.) or genetic diseases. T-lymphocyte replacement also concerns organ or cell (allogeneic, or even xenogeneic) transplantations, as well as all pathologies for which the reinjection of modified lymphocytes may be recommended, for example, in order to secrete a therapeutic protein. This system may also be advantageous in order to control the immune response against a therapeutic protein administered, for example, to correct a genetic deficiency of that protein. One of the drawbacks in this situation is that, because of the genetic deficiency, the patient recognizes that protein as foreign and rejects it by an immune response. According to the principles of the invention, if the

T-lymphocytes express a suicide gene, it is then possible to control that harmful immune response.

Thus, autoimmune diseases, also called systemic diseases (for example, Disseminated Lupus Erythematosus, Rheumatoid Polyarthritis, Polymyositis, etc.) are diseases having a clear immunological component, as proven by the biological and histological research performed on patients. For many of these pathologies, the *primum movens* is unknown and the origin of the pathology appears to be multifactorial. Nevertheless, the central element continues to be a maladjusted immune response. Furthermore, in these pathologies it is generally possible to define the period during which the T-Ly's responsible for the pathology are activated and therefore undergoing division. Consequently, the administration of the prodrug during this phase, according to this invention, allows these cells to be the ones mainly affected. In addition, this strategy leads to the deletion of the clones of the cells responsible for the pathology, and thus, in principle, to a lasting, or even permanent, immunosuppression in the absence of the central T-Ly production.

For example, in a particular embodiment of the invention, a patient suffering from rheumatoid polyarthritis receives, after his or her own T-lymphocytes have been depleted, an administration of a composition consisting of his or her own T-lymphocytes, genetically modified to express a suicide gene (an autologous composition) or allogeneic T-lymphocytes, whether or not modified. Then, during possible attacks of rheumatoid polyarthritis occurring after administration, treatment with a drug transformed into a toxic metabolite by the suicide gene allows the reactive clones to be destroyed and so to suppress the immunopathological response.

This invention may also be used for treating virally induced immunopathologies. The immune response against infectious agents can often have immunopathological consequences which sometimes go as far as death. The most classic example is that of the response to certain viruses responsible for hepatitis. These viruses replicate into hepatocytes, and it is the destruction of the infected hepatocytes by the immune system that leads to a sometimes fatal hepatitis. In other situations, the host's immune response seems incapable of eradicating the virus, and, on the contrary, participates in maintaining a chronic hepatitis. This is well illustrated by the evolution of the so-called C-virus hepatitis. Although approximately two-thirds of patients are able to eliminate the virus, the remaining third develops a chronic hepatitis. The evolution of this chronic hepatitis is independent of the viral replication rate and is, on the contrary, accompanied by biological signs that are witnesses of a dysimmune response (for example, the frequent presence of anti-DNA antibodies or of a cryoglobulinemia). This invention allows the elimination of the active T-lymphocyte clones responsible for the immunopathology, and thus the reduction of the consequences of the infection in the host.

On the other hand, the synthesis of IgE's involved in certain allergies is also controlled by T-lymphocytes. In addition, the production of B-lymphocytes is realized through immature bone marrow cells, and these cells have a much shorter half-life than T-lymphocytes. Consequently, it is also possible, by using the T-lymphocyte exchange technique according to the invention, to control allergic responses, more generally antibody responses, by controlling the T-responses.

This invention may also be used for treating or preventing organ transplant rejection.

The standard treatment for a certain number of organ pathologies is, when necessary, to replace that organ with a healthy organ from a deceased donor (or from a living donor in certain cases, even of another species). Even though rigorous care is taken in selecting organ donors with maximum compatibility with respect to histocompatibility antigens, except for situations of transplants between homozygous twins, organ transplantation always leads to the development of an immune response directed against the antigens specifically expressed by that organ. In spite of the immunosuppressive treatments implemented, this reaction often leads to the rejection of the transplanted organ (it is the main cause of allogeneic transplant failure). Setting aside certain hyperacute or acute rejections that involve essentially humoral responses, most of the time, organ transplant rejection is essentially mediated by T-lymphocytes.

Numerous teams have developed modified or unmodified allogeneic or xenogeneic cell transplantation projects, in order for them to produce a factor for a therapeutic purpose (for example, cells from pancreatic β islets, fibroblasts, etc.). In particular, it has been proposed for diseases as diverse as diabetes, Parkinson's disease, and even in organoid genetic therapy. The main obstacle to these transplantations continues to be the rejection of allogeneic or xenogeneic cells. In order to compensate for this drawback, numerous devices have been proposed to separate the transplanted cells from the immune system. These systems range from microencapsulation to the insertion of cells in porous or semipermeable materials, etc. Unfortunately, none of these systems has proven to be effective enough to be proposed for clinical use.

If, in addition, these patients are in good immunological health, it is legitimate to consider the standard T-lymphocyte exchange while preserving a stock of lymphocytes from the patients in case a problem occurs later.

Now this invention offers a new medical approach to promote the success of organ or cell transplantations. In particular, a T-lymphocyte exchange is performed in the recipient subject which, if needed, allows any possible development of an immune response against the transplant to be controlled, by the selective destruction of alloreactive T-lymphocytes. Within the framework of organ or cell transplantations, it is possible to apply these therapies, either by reimplanting an organ or cells and allogeneic T-lymphocytes from the same donor in the recipient (which therefore tolerate the transplanted organ or cells), or by transplanting an organ

or cells from an allogeneic donor and reimplanting the patient's own genetically modified T-lymphocytes in order to control the response against that organ or those cells. Finally, it is possible to consider transplanting an organ or cells and allogeneic T-lymphocytes from two different donors.

One particularly advantageous embodiment of this invention consists of treating immunopathologies within the framework of organ or cell transplantations. More particularly, the treatment represents the total or partial prevention, or the reduction or suppression of immunopathologies mediated by T-lymphocytes, which are responsible for transplantation failures.

Similarly, the invention may also be implemented by treating (preventing, reducing or totally suppressing, for example) graft-versus-host disease (GVHD). Bone marrow transplantation is a standard treatment in a large number of clinical situations, especially for many leukemias. Conventional treatment therefore depends on donor conditioning, its purpose being to eliminate the maximum number of tumor cells, and its consequence being the creation of a medullary aplasia requiring bone marrow transplantation. This bone marrow transplantation is preferably performed with allogeneic cells, because they have proven that they may be responsible for an effect called "graft-versus-leukemia" that considerably limits the relapse rate of the leukemias thus treated. Unfortunately, the allogeneic T-lymphocytes are also responsible for the graft-versus-host disease, which is always serious and sometimes fatal. The use of this invention allows the destruction of the T-cells responsible for GVH, and therefore the treatment of this immunopathology.

Other important examples of pathologies that can be treated by replacing T-lymphocytes are HIV infections or certain genetic diseases.

Thus, T-lymphocytes are a main target of infection by HIV, whose clinical evolution depends in great part on the level of these lymphocytes in the infected patient. Certain therapeutic strategies for HIV infection are based on the transfer of T-lymphocytes genetically modified to resist the virus. However, there are also patients whose T-lymphocytes are less susceptible to HIV infection because of spontaneous mutations in the genes coding for the proteins involved in the replication cycle of the virus. Thus, in the same way as the response to the hepatitis virus, there are genetic differences expressed by the T-lymphocytes, which make them more or less sensitive to an HIV infection. This invention allows advantage to be taken of these very diverse genetic factors, especially for replacing the T-lymphocytes of a subject who is sensitive to a given pathology by those of a subject who is not sensitive to it.

In addition, there are many genetic diseases that are linked to the inability to synthesize a protein which, if synthesized by certain specific cellular types (not necessarily T-lymphocytes), nevertheless has a general action. Thus, these pathologies can often be treated by bone marrow

transplantation when a compatible donor can be found. These same diseases could be treated by transferring T-lymphocytes (muccopolysaccharidose, etc.).

There are also genetic diseases that specifically affect lymphocytes (adenosine deaminase deficit, etc.), which can also be treated by the standard T-lymphocyte exchange according to the invention. Finally, possible radiation accidents or any other acute medullary insufficiency (for example, toxic, drug-related, etc.) can also be handled by a T-lymphocyte exchange according to the invention. Thus, hematopoietic stem cell banks and T-lymphocyte banks from the same donors can be established, for whom alone the T-lymphocytes have undergone a genetic modification allowing the immunopathological reactions to be controlled. Thus, "universal banks" are available, which allow bone marrow transplantations with mature T-lymphocytes for situations that require them.

According to another variant of the invention, the population of the donor's T-lymphocytes may be used to prevent or treat the aging of immune functions, such as anergies.

This invention shall be described in greater detail with the aid of the following examples, which should be considered as illustrative, not limiting.

Figure captions

Figure 1: Schematic representation of the Standard T-Lymphocyte Exchange principle.

Figure 2: Sensitivity to GCV of T-cells expressing a thymidine kinase. The splenocytes were cultured 2 days in the presence of ConA and of increasing concentrations of GCV, then loaded overnight with titrated TdR (a 100% uptake was observed in the absence of GCV). The triangles, squares and circles correspond to different forms of TK. The upper curve corresponds to nontransduced T-lymphocytes.

Figure 3: GHV protective effect of a 7-day treatment by GCV. The radiation control group is composed of nontransplanted irradiated mice (n=15). A GVH that is fatal for 100% of the mice on D35 is induced by the coinjection of bone marrow cells and allogeneic T-Ly's (n=25). The survival of the treated mice (n=13) is compared to that obtained in mice receiving only the bone marrow and therefore not developing GVH (n=15).

Figure 4: Pharmacogenetic control of lymphocytic choriomeningitis in mice expressing the suicide gene in CD4 and CD8 lymphocyte populations. FVB or C57BL/6 mice were irradiated and reconstituted by syngeneic bone marrow from FVB mice or transgenic EPDTKL20 mice, or by allogeneic bone marrow. After reconstituting the immune system, the recipients were infected intracerebrally by the lymphocytic choriomeningitis virus (10^4 pfu stock Arm/53b LCMV) and treated with GCV for 7 days (twice daily intraperitoneal 100 mg/kg/d injections). The mice expressing the TK gene in their lymphocytes are protected, survive, have eliminated the LCMV and have high anti-LCMV antibody strength.

Examples

1. Study of immunopathological response regulation

In order to study the possibility of controlling pathological immune responses with the help of suicide genes, we developed transgenic mice expressing the HSV1-TK gene in T-Ly's. This expression is obtained with the aid of regulating sequences derived from the gene coding for the CD4 molecule, having previously proven that they lead to the specific expression of a transgene in mature CD4⁺ and CD8⁺ T-lymphocytes, except for immature CD4⁻ CD8⁻ or CD4⁺ CD8⁺ thymocytes [26]. In vitro transgenic mice T-Ly's are effectively destroyed by GCV (Figure 2).

In all of the following examples, the T-lymphocytes come from transgenic mice only in order to simplify the experimental procedure. The same experiments may be reproduced taking T-lymphocytes from mice or human beings in which the same suicide gene can be transferred by a retroviral vector according to good practice.

1.1. GVH control in mice

By using transgenic mice expressing the HSV1-TK gene in the T-lymphocytes, we proved that it is perfectly possible to control the allogeneic response responsible for the graft-versus-host reaction in those animals. We have therefore proven the principle of this capacity for controlling the T-lymphocytes responsible for the GVH. Moreover, it should be mentioned that this therapeutic efficacy can be obtained by extremely short gancyclovir treatments, and that we have proven that, at the end of these treatments, not only had the pathology been prevented, but the mice had a normal immune response.

We used transgenic mice as HSV1-TK⁺ T-Ly donors for developing a graft-versus-host (GVH) disease model after allogeneic bone marrow transplantation (BMT) [8]. Thus, in lethally irradiated mice, an allogeneic BMT depleted of mature T-Ly's led to a survival of all of the animals and to complete hematopoietic reconstitution by the donor cells. On the other hand, the T-Ly supplemented transplantation led to a 100% mortality of the animals, which presented the clinical and histological symptoms of GVH. In this model, we proved that if the T-Ly's express the HSV1-TK suicide gene, a GCV treatment instituted the day of the transplantation and extended for 7 days allows the prevention of clinical GVH (Figure 3). When the animals are sacrificed, the histological analysis objectifies the absence of any histological sign of GVH in these animals. The treatment is equally effective on a declared GVH.

The cells of hematopoietic origin found in these animals come from the donor. Interestingly, we observed that the T-Ly's that survive the GCV treatment are still able to respond in vitro to stimuli by a mitogen or a third-party alloantigen, but not by cells from the

donor or the recipient. These results proved that a GCV treatment allows the elimination of alloreactive T-Ly clones, while preserving a compartment of T-Ly's capable of responding to other antigenic stimuli.

1.2. Treatment of autoimmune diabetes in diabetic mice (NOD) by T-lymphocyte exchange: proof of principle

The purpose of this experiment is to prove the efficacy of genetically modified T-lymphocyte exchange in controlling a spontaneous autoimmune disease (AID), here represented by the NOD autoimmune diabetic mouse model. The T-lymphocyte exchange is performed under conditions similar to those considered for clinical practice. The T-lymphocyte exchange is performed with T-lymphocytes that are allogeneic to the recipient, allowing the destruction of possible residual autoimmune cells. The T-lymphocyte exchange is performed with genetically modified T-lymphocytes expressing the TK suicide gene. In this model, GVH control is based on the use of genetically modified T-lymphocytes expressing the TK gene, associated with a gancyclovir (GCV) treatment. This system allows the destruction of the dividing TK T-lymphocytes, thus controlling the GVH. The T-lymphocyte exchange is performed after a nonmyeloablative conditioning of NOD mice by irradiating at 8-9 Gy, leading to 100% survival of such mice on D 120. The T-lymphocyte exchange is performed by injecting 10^7 cells in the retroorbital sinus of irradiated mice, associated with a 7-day preventive treatment with gancyclovir, initiated at the same time as transplantation. A first control group is composed of mice receiving 10^7 cells of allogeneic bone marrow, but no T-lymphocytes. In these mice, the transplant may be partially or totally rejected, and the AID may reappear. A second control group is composed of mice receiving 10^7 bone marrow cells from NOD mice, but no T-lymphocytes.

The efficacy of the strategy is evaluated by:

- analyzing the presence of genetically modified T-lymphocytes characterized by the expression of donor origin CMH molecules;
- determining clinical signs of autoimmune diabetes (blood sugar above 2 g/L);
- the presence of lymphocytic infiltrates in the Langerhans islets.

Results

· CSMNSP origin

Table 1: Study of chimerism 180 days after transplantation

		① Lymphocytes T d'origine du donneur
②	Contrôle NOD	2 0/2
③	NOD irradi. sublétalement	3 0/3
④	NOD greffées syng.	6 0/6
⑤	NOD greffées et protégées	3 3/3

Key: 1 Donor origin T-lymphocytes
 2 NOD control
 3 Sublethally irradiated NOD's
 4 Syngeneically transplanted NOD's
 5 Transplanted and protected NOD's

180 days after transplantation, hematopoietic reconstitution is evaluated by CSMNSP marking with monoclonal Ac's, and analyzed by flow cytometry. The donor-origin B- and T-cells are determined by the expression of CMH class I molecules (H-2^q). The recipient's B- and T-cells are determined by the expression of CMH class II molecules (J-A^k).

In the NOD mice in which a T-lymphocyte exchange was performed, only the donor origin cells are present.

· Presence of lymphocytic infiltrates after the T-lymphocyte exchange

Table 2: Periinsulite and insulite in NOD mice after T-lymphocyte exchange

	n	Présence d'infiltrats lymphocytaires ① Langerhansiens
② NOD greffées syng.	4	4/4
③ NOD greffées et protégées	3	0/3

Key: 1 Presence of Langerhans lymphocytic infiltrates
 2 Syngeneically transplanted NOD's
 3 Transplanted and protected NOD's

180 days after transplantation, the pancreas of the mice is removed, set in Bouin liquid, and then placed in paraffin. The presence of lymphocytic infiltrates is evaluated on 4 micron cuttings after hematoxylin and eosin marking.

No lymphocytic infiltrates were observed in any mouse pancreas subjected to a T-lymphocyte exchange.

In addition, the mice remained normoglycemic, thus proving the absence of diabetes.

In conclusion, the recipient mice are reconstituted by the allogeneic T-lymphocytes and no longer develop the autoimmune disease.

1.3. Control of a virally induced immunopathology

We wished to apply the same system to controlling an immunopathological reaction induced by a virus. The most classic experimental system is that of controlling CML virus-induced encephalopathy in mice.

The immune response to the CML virus is well known. Thus, the animals intracerebrally inoculated with this virus die very quickly from a brain complication mediated by cytotoxic T-lymphocytes. In animals that have received prior immunization, the sensitized T-lymphocyte reactivity kinetics is accelerated, leading to the protection and survival of the mice. In animals receiving gancyclovir at the time of the intracerebral injection, the destruction of cytotoxic

T-lymphocytes activated by viral replication leads to control of the brain complication (Figure 4).

1.4. Control of transplant rejection

We then used our mice to try to control immune responses responsible for organ transplant rejection. The model used is the one that may be considered the most physiological. It is a heart transplant vascularized in a heterotopical position in mice. When this allogeneic transplantation is performed in untreated mice, rejection usually occurs within several days of transplantation. When the mice are treated with gancyclovir during a very short period (7 days beginning on the day of transplantation), the heart is tolerated permanently and retains its vital functions as proven by its beating.

2. Therapeutic T-lymphocyte exchange protocol applied to autoimmune pathologies.

Treatment begins with a T-lymphocyte ablation. This ablation can be performed, for example, by combining the administration of "antilymphocytic serum" (immunoglobulins capable of recognizing T-lymphocytes and destroying them in the presence of the patient's complement, that are usually produced in rabbits, for example, by the Mérieux laboratories). The amount of this ALS administered and the duration of the administration depend on the clinical data of the pharmaceutical lot and the manufacturer's recommendations. This treatment may be performed at the same time as the administration of other immunosuppressants, such as cyclophosphamide and cyclosporin. Finally, in order to reach the tissular T-lymphocytes that might be more resistant to these treatments, it is possible to add radiation delivered while protecting the patient's bone marrow.

At the end of this treatment, the T-lymphocyte deletion may be analyzed at the peripheral blood level by their standard count. A peripheral blood lymphocyte deletion greater than 90% is preferable.

At the end of this treatment, an intravenous injection of T-lymphocytes is performed in the patients at a dosage rate that preferably ranges between 10^9 and 10^{11} cells. In this case the T-lymphocytes have been obtained from an allogeneic donor having maximum compatibility of the main histocompatibility antigens. This may be, for example an identical HLA cousin, for situations in which the genetic risk of finding the same pathologies within the family is not very high. The reinjected cells have been previously transduced by a suicide gene such as the HSV1-TK gene, and the re-injected cells contain at least 90% transduced cells. In addition, all of the quality controls are performed (phenotype, repertoire analysis).

In the first few days after injection, preferably during the week following the injections, 48 h after injection, a gancyclovir treatment is administered to the patient at a rate of 10 mg/kg

twice daily. If signs of GVH should occur earlier, treatment may be considered even earlier. The treatment lasts at least one week. At the end of this treatment, follow-up is performed on the patient, especially to verify T-lymphocyte reconstitution.

Follow-up is also performed on signs indicating a graft-versus-host reaction, such as, for example, skin problems. If such manifestations occur, a curative gancyclovir treatment is undertaken.

Treatment is repeated and finished when the lymphocyte count returns to values of at least 200 T-lymphocytes per mm^3 .

If serious side effects occur during treatment, such as, for example, a GVH that cannot be controlled by gancyclovir, it is possible to retreat the patient with medications allowing the destruction of the T-lymphocytes, in order to reinject his or her own cells, which have been previously cryopreserved. In addition, since these T-lymphocytes are built with a membrane marker, an injection of antimarker antibodies helps to eliminate the T-lymphocytes, if necessary.

For other types of autoimmune pathologies, the reinjected lymphocytes may be the patient's lymphocytes, similarly transduced by the HSV1-TK gene. Under these conditions, at the end of cell reinjection, no gancyclovir treatment is instituted. After ascertaining the patient's T-lymphocyte pool reconstitution, when acute pathology attack phases occur, a gancyclovir treatment is instituted as soon as possible after the identification of these rises, which could be, for example, attacks of inflammatory arthritis in the treatment of a rheumatoid arthritis, or neurological or muscular problems in the case of multiple sclerosis.

3. Therapeutic T-lymphocyte exchange protocol applied to organ transplant treatment.

In this therapeutic indication, the patient is conditioned as described above. He or she is then reconstituted, for example, with his or her own cells expressing the suicide gene in the absence of gancyclovir treatment. When the patient has reconstituted his or her immune system, the organ transplant itself may be undertaken. Immediately after transplantation, a prophylactic treatment with gancyclovir lasting approximately one week may be undertaken. The patient is then monitored for the evolution of clinical signs that might reveal the beginning of transplant rejection. It is indicated that standard immunosuppressive treatments, such as, for example, cyclosporin, may be used in this situation.

In another therapeutic mode, the lymphocytes may come from the organ donor. In this case, they will tolerate the transplant and the operating mode is therefore a GCV treatment immediately after the administration of the genetically modified T-lymphocytes in order to suppress the GVH.

4. Therapeutic protocol for preventing immunological aging.

The immune system is subject to aging, which is characterized by a poor regulation of the immune response, such as the appearance of autoimmune diseases or the inability to mount an effective immune response against a new antigen.

In this application, treatment is preventive. When the patient is still young, T-lymphocytes are obtained from him or her. It is easy and safe to obtain 10^8 lymphocytes during a cytopheresis.

This operation may be repeated fairly frequently at time intervals of several months.

It is currently accepted that the so-called "memory" T-lymphocyte subpopulations are circulating. If this is not the case, they will have to be removed surgically or after they are mobilized by one or another growth factor.

In the preferred embodiment, the T-lymphocytes are cultured and extended until they reach $10^{10} - 10^{11}$ T-lymphocytes.

The cells are then distributed and frozen for several years. When the need arises, by the appearance of an immunological problem or difficulty in producing an immune response, the T-lymphocytes are thawed and reinjected in the subject.

This reinjection may apply to all or part of the frozen T-lymphocytes, and may be preceded by a total or partial ablation of the circulating T-lymphocytes.

The replacement, for example, of only one subclass of T-lymphocyte by another subclass of T-lymphocytes may be planned. For example, it is perfectly possible to destroy only CD8 lymphocytes (or those bearing a particular TCR) and to select the previously frozen T-lymphocytes in order to inject only the desired T-lymphocyte subclass, or even to selectively deplete a given T-lymphocyte subclass from the transplant.

Thus the patient's T-lymphocytes are replaced by younger and better T-lymphocytes in order to ensure their functions. In the case of autoimmune patients, the alloreactive T-lymphocytes are destroyed and replaced by safe T-lymphocytes. In the case of a pathology connected to the aging of the T-lymphocyte population, it is a preventive therapy. In effect, it is truly a matter of "rejuvenating" the T-lymphocytes and therefore of the immune system.

Bibliography

- [1] P. Tiberghien, C.W. Reynolds, J. Keller, S. Spence, M. Deschaseaux, J.M. Certoux, E. Contassot, W.J. Murphy, R. Lyons, Y. Chiang, P. Hervé, D.L. Longo, F.W. Ruscetti, Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: an approach for specific in vivo donor T-cell depletion after bone marrow transplantation?, *Blood* 84 (1994) 1333-1341.
- [2] C. Bordignon, L.D. Notarangelo, N. Nobili, G. Ferrari, G. Casorati, P. Panina, E. Mazzolari, D. Maggioni, C. Rossi, P. Servida, a.l. et, Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients, *Science* 270 (1995) 470-475.
- [3] Anonymous, Human gene marker/therapy clinical protocols, *Hum. Gene Ther.* 8 (1997) 1499-1530.
- [4] F. Moolten, Tumor sensitivity conferred by inserted herpes thymidine kinase genes: paradigm for prospective cancer control strategy., *Cancer Res* 46 (1986) 5276-5281.
- [5] K. Culver, Z. Ram, S. Wallbridge, H. Ishii, E. Oldfield, R. Blaese, *In vivo* gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors., *Science* 256 (1992) 1550-1552.
- [6] M. Caruso, Y. Panis, S. Gagandeep, D. Houssin, J. Salzmänn, D. Klatzmänn, Regression of established macroscopic liver metastases after *in situ* transduction of a suicide gene., *Proc Natl Acad Sci USA* 90 (1993) 7024-7028.
- [7] C. Bonini, G. Ferrari, S. Verzeletti, P. Servida, E. Zappone, L. Ruggieri, M. Ponzoni, S. Rossini, F. Mavilio, C. Traversari, C. Bordignon, HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia [see comments], *Science* 276 (1997) 1719-1724.
- [8] J.L. Cohen, O. Boyer, B. Salomon, R. Onclercq, F. Charlotte, S. Bruel, G. Boisserie, D. Klatzmänn, Prevention of graft-versus-host disease in mice using a suicide gene expressed in T lymphocytes, *blood* 89 (1997) 4636-4645.

- [9] M. Helene, V. Lake-Bullock, J.S. Bryson, C.D. Jennings, A.M. Kaplan, Inhibition of graft-versus-host disease. Use of a T cell-controlled suicide gene, *J Immunol* 158 (1997) 5079-5082.
- [10] F.L. Moolten, Drug sensitivity ("suicide") genes for selective cancer chemotherapy, *Cancer Gene Ther* 1 (1994) 279-287.
- [11] J.A. Fyfe, P.M. Keller, P.A. Furman, R.L. Miller, G.B. Elion, Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl)guanine., *J Biol Chem* 253 (1978) 8721-8727.
- [12] Y. Nishiyama, F. Rapp, Anticellular effects of 9-(2-hydroxyethoxymethyl) guanine against herpes simplex virus-transformed cells, *J. Gen. Virol.* 45 (1979) 227-230.
- [13] P.A. Furman, P.V. McGuirt, P.M. Keller, J.A. Fyfe, G.B. Elion, Inhibition by acyclovir of cell growth and DNA synthesis of cells biochemically transformed with herpesvirus genetic information, *Virology* 102 (1980) 420-430.
- [14] R.L. Davidson, E.R. Kaufman, C.S. Crumpacker, L.E. Schnipper, Inhibition of herpes simplex virus transformed and nontransformed cells by acycloguanosine: mechanisms of uptake and toxicity, *Virology* 113 (1981) 9-19.
- [15] G.B. Elion, The biochemistry and mechanism of action of acyclovir., *J Antimicrob Chemother* 12 Suppl B (1983) 9-17.
- [16] A.K. Field, M.E. Davies, C. DeWitt, C. Perry, H.C. Liou, R. Germershausen, J. Karkas, W.T. Ashton, D.B. Jonhston, R.L. Tolman, 9-([2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]guanine: a selective inhibitor of herpes group virus replication., *Proc Natl Acad Sci USA* 80 (1983) 4139-4143.
- [17] S. Oliver, G. Buble, C. Crumpacker, Inhibition of HSV-transformed murine cells by nucleoside analogs, 2'-NDG and 2'-nor-cGMP: mechanisms of inhibition and reversal by exogenous nucleosides, *Virology* 145 (1985) 84-93.

[18] M.H. St. Clair, C.U. Lambe, P.A. Furman, Inhibition by ganciclovir of cell growth and DNA synthesis of cells biochemically transformed with herpesvirus genetic information, *Antimicrob Agents Chemother* 31 (1987) 844-849.

[19] L.W. Bi, In vitro evidence that metabolic cooperation is responsible for the bystander effect observed with HSV tk retroviral gene therapy, *Hum Gene Ther* 4 (1993) 725-731.

[20] F.L. Moolten, J.M. Wells, R.A. Heyman, R.M. Evans, Lymphoma regression induced by ganciclovir in mice bearing a herpes thymidine kinase transgene, *Hum Gene Ther* 1 (1990) 125-134.

[21] Z. Ezzeddine, R. Martuza, D. Platika, M. Short, M. Malick, A. Choi, X. Breakefield, Selective killing of glioma cells in culture and *in vivo* by retrovirus transfer of the herpes simplex virus thymidine kinase gene., *New Biol* 3 (1991) 608-614.

[22] D. Barba, J. Hardin, R. Jasodhara, F. Gagae, Thymidine kinase-mediated killing of rat brain tumors., *J Neurosurg* 79 (1993) 729-735.

[23] S. Chen, H. Shine, J. Goodman, R. Grossman, S. Woo, Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer *in vivo*., *Proc Natl Acad Sci USA* 91 (1994) 3054-3057.

[24] S.M. Freeman, C.N. Abboud, K.A. Whartenby, C.H. Packman, D.S. Koeplin, F.L. Moolten, G.N. Abraham, The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified, *Cancer Res* 53 (1993) 5274-5283.

[25] Z. Ram, K.W. Culver, E.M. Oshiro, J.J. Viola, H.L. DeVroom, E. Otto, Z. Long, Y. Chiang, G.J. McGarrity, L.M. Muul, D. Katz, R.M. Blaese, E.H. Oldfield, Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells [see comments], *Nat Med* 3 (1997) 1354-1361.

[26] P. Salmon, O. Boyer, P. Lores, J. Jami, D. Klatzmann, Characterization of an intronless CD4 minigene expressed in mature CD4

and CD8 T cells, but not expressed in immature thymocytes, J. Immunol. 156 (1996) 1873-1879.

[27] C. Bordignon, C. Bonini, S. Verzeletti, N. Nobili, D. Maggioni, C. Traversari, R. Giavazzi, P. Servida, E. Zappone, E. Benazzi, M. Bernardi, F. Porta, G. Ferrari, F. Mavilio, S. Rossini, R.M. Blaese, F. Candotti, Transfer of the HSV-tk gene into donor peripheral blood lymphocytes for in vivo modulation of donor anti-tumor immunity after allogeneic bone marrow transplantation, Hum Gene Ther 6 (1995) 813-819.

[28] P. Tiberghien, J.Y. Cahn, A. Brion, E. Deconinck, E. Racadot, P. Herve, N. Milpied, B. Lioure, E. Gluckman, P. Bordignon, W. Jacob, Y. Chlang, S. Marcus, C. Reynolds, D. Longo, Use of donor T-lymphocytes expressing herpes-simplex thymidine kinase in allogeneic bone marrow transplantation: a phase I-II study, Hum Gene Ther 8 (1997) 615-624.

Claims

1. Method for replacing a subject's T-lymphocytes, consisting of the administration of a specific population of T-lymphocytes.

2. Method for replacing a T-lymphocyte population of a subject, consisting of the depletion of the subject's T-lymphocytes and the administration of a specific T-lymphocyte population.

3. Method according to Claim 2, consisting of the administration of a population of modified T-lymphocytes.

4. Method according to Claim 1 or Claim 3 in which the T-lymphocyte population administered consists of T-lymphocytes that are autologous or syngeneic in relation to the subject.

5. Method according to Claim 1 or Claim 3 in which the T-lymphocyte population administered consists of T-lymphocytes that are allogeneic in relation to the subject.

6. Method according to Claim 3 in which the T-lymphocyte population administered consists of genetically modified T-lymphocytes.

7. Method according to Claim 6 in which the modified T-lymphocytes consist of a nucleic acid coding for a toxic product.

8. Method according to Claim 7 in which the nucleic acid codes for a protein having a conditional toxicity.

9. Method according to Claim 8, in which the nucleic acid codes for thymidine kinase.
10. Method according to Claim 6 in which the modified T-lymphocytes consist of a nucleic acid coding for a therapeutic product.
11. Method according to Claim 6 in which the modified T-lymphocytes are genetically modified by means of a viral vector.
12. Method according to Claim 10 in which the viral vector is a retroviral or AAV vector.
13. Method according to Claim 3 in which the population of modified T-lymphocytes consists of immunologically modified T-lymphocytes.
14. Method according to Claim 13 in which the immunological modification consists of the modification of the repertoire of modified T-lymphocytes.
15. Method according to Claim 3 in which the population of modified T-lymphocytes consists of T-lymphocytes giving increased immunity.
16. Method according to Claim 6 in which the population of modified T-lymphocytes consists of T-lymphocytes giving increased immunity.
17. Method according to Claim 12 in which the population of modified T-lymphocytes consists of T-lymphocytes giving increased immunity.
18. Method according to Claim 2 in which the T-lymphocyte depletion stage consists of the administration of one or more immunosuppressive agents to the subject.
19. Method according to Claim 18 in which the T-lymphocyte depletion consists of administering a T-lymphocyte-specific immunosuppressive agent to the subject.
20. Method according to Claim 18 in which the immunosuppressive agent is an anti-CD3, CD4 and/or CD8 antibody or serum.
21. Method according to Claim 8 consisting, in addition, of the administration to the subject of a drug that can be transformed into a toxic metabolite by the protein.
22. Composition consisting of:
 - one or more immunosuppressive agents, and
 - a composition consisting of a T-lymphocyte population.
23. Composition according to Claim 22, consisting of:
 - a T-lymphocyte population consisting of a suicide gene,
 - a drug that can be transformed into a toxic metabolite by the suicide gene.
24. Procedure for preparing a composition of modified T-lymphocytes consisting of:
 - taking a sample of T-lymphocytes from a subject, and
 - creating a reconstitution of the repertoire in those lymphocytes and/or subpopulations of T-lymphocytes.
25. Procedure according to Claim 24 in which the reconstitution of the repertoire is performed by depleting T-lymphocytes specific to antigens involved in pathologies.

26. Method according to Claim 1 or Claim 3, for administering a T-lymphocyte population to a subject who is suffering from or is susceptible to suffering from an immunopathology.

27. Method according to Claim 26 in which the subject has undergone organ or cell transplantation.

28. Method according to Claim 1 or Claim 3, for administering a T-lymphocyte population to a subject who is suffering from or is susceptible to suffering from cellular immune system aging.

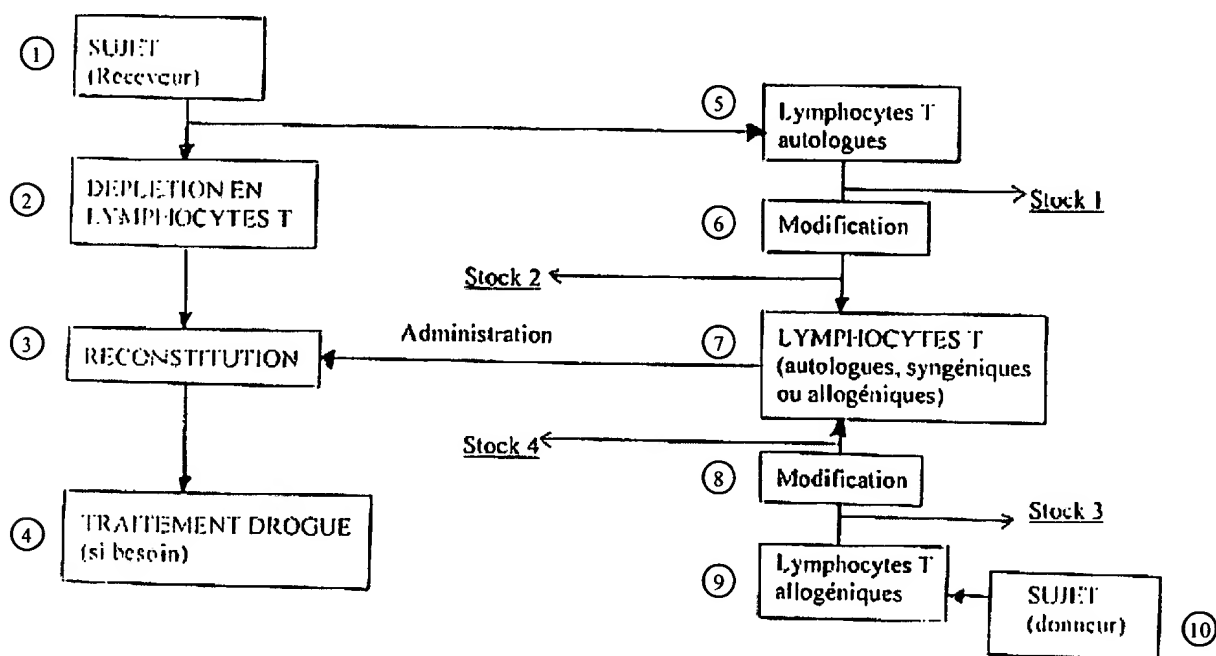


Figure 1

T-lymphocyte exchange procedure

- Key:
- 1 Subject (recipient)
 - 2 T-lymphocyte depletion
 - 3 Reconstitution
 - 4 Drug treatment (if necessary)
 - 5 Autologous T-lymphocytes
 - 6 Modification
 - 7 T-lymphocytes (autologous, syngeneic or allogeneic)
 - 8 Modification
 - 9 Allogeneic T-lymphocytes
 - 10 Subject (donor)

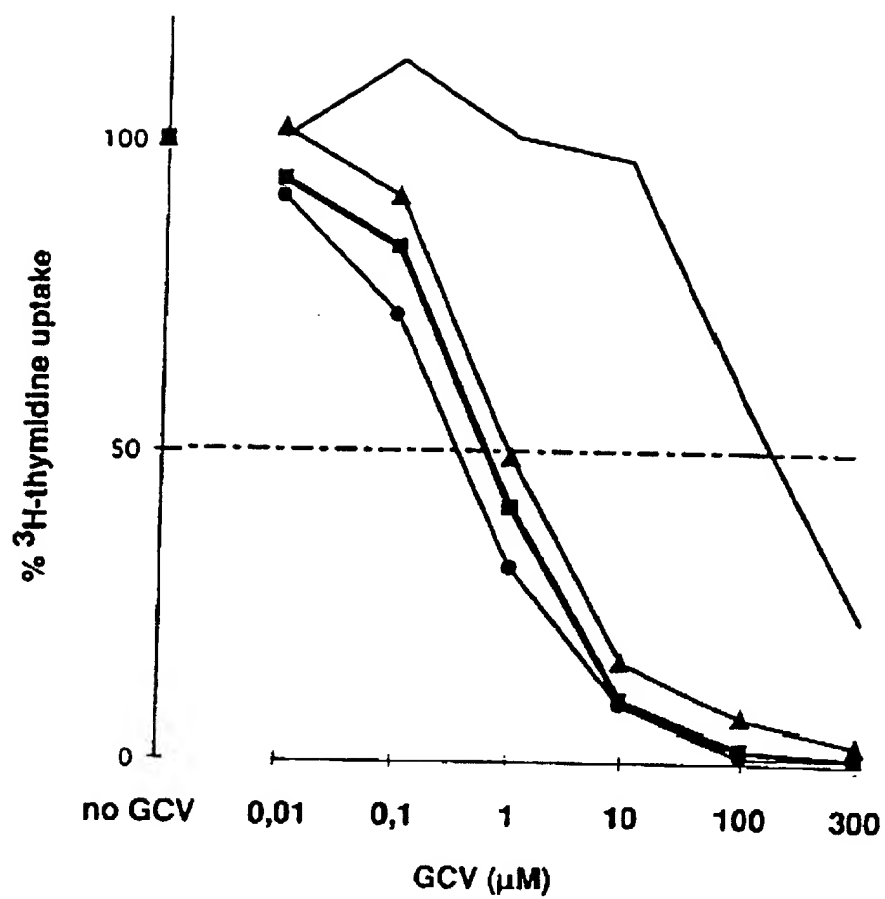


FIGURE 2

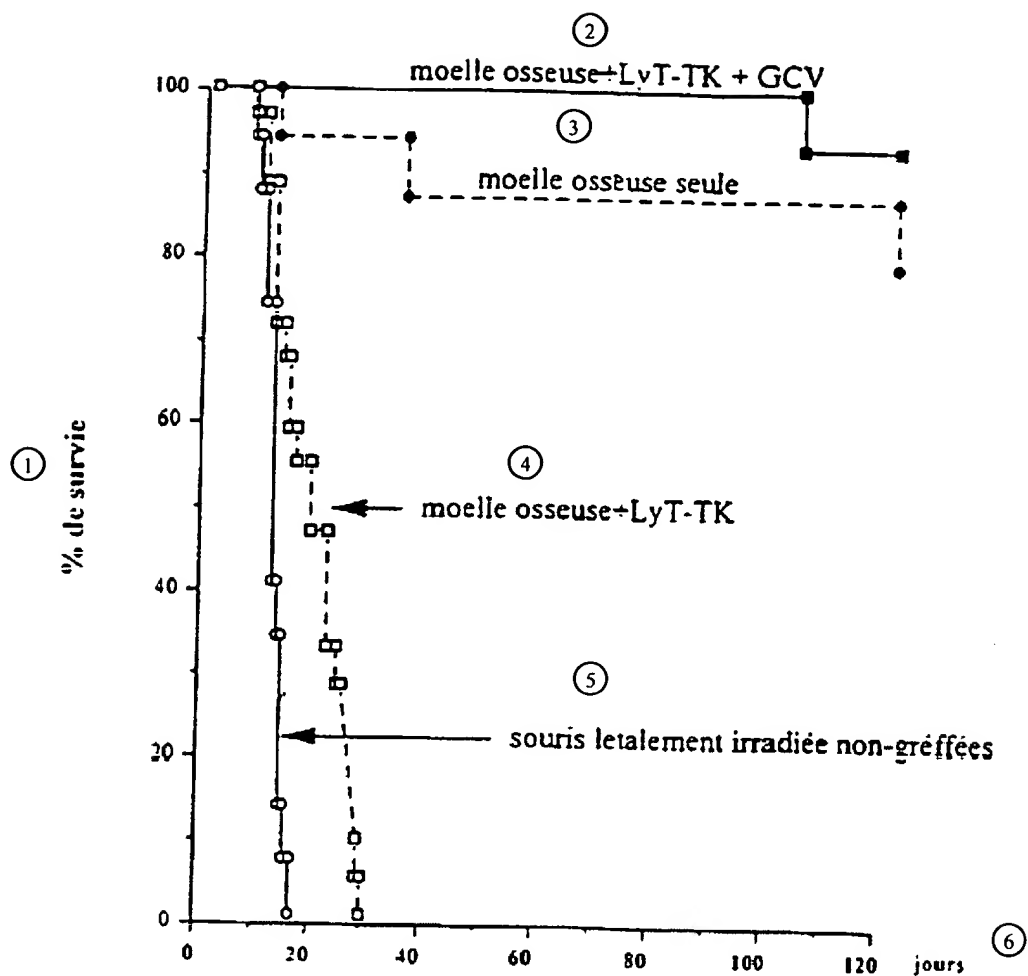


FIGURE 3

Key: 1 % survival
 2 Bone marrow + TK-T-Ly + GCV
 3 Bone marrow alone
 4 Bone marrow + TK-T-Ly
 5 Nontransplanted lethally irradiated mice
 6 Days

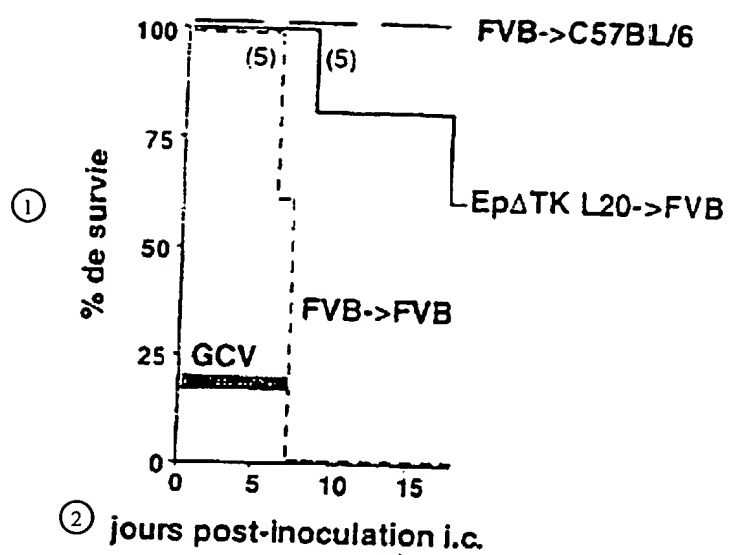


FIGURE 4

Key: 1 % survival
2 Days post-i.c. inoculation